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A NEW MEDIUM FOR THE DETECTION OF UREA-SPLITTING ORGANISMS[1]

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Bacteriological laboratories engaged in diagnostic or research problems concerning pathogenic enteric bacilli, namely, the *Salmonella* and *Shigella* groups, invariably find that members of the genus *Proteus* are by far the most misleading. A dependable, easily interpreted, routine differential test medium for screening the latter group of bacilli becomes desirable. Urease production is a characteristic activity of the genus *Proteus* (Bergey *et al.*, 1939). The hydrolysis of urea by urease has been described by Werner (1923) as "an 'alkaline fermentation' during which the 'carbonate of ammonia' was formed." Rustigian and Stuart (1941) recommended a urea medium for the detection of this enzyme activity. A modification of the latter medium has been described by Anderson (1945). Since one of the products of the splitting of urea is the formation of ammonia, Rustigian and Stuart suggested that phenol red indicator be employed to detect this alkaline change colorimetrically. Howell and Sumner (1934) reported that the pH optimum for urease activity upon 2.5 per cent urea is 6.9 with phosphate buffer.

The present report describes a new medium for the detection of urea-splitting organisms. Colorimetric changes are sharp and permit easy interpretation of results.

METHODS AND MATERIALS

I. The medium is prepared in three parts:

A. The buffered semisolid "deep,"

Bacto tryptose (Difco)	10.0 g
Sodium chloride	5.0 g
Bacto agar (Difco)	3.0 g
Dipotassium phosphate	1.5 g
Monopotassium phosphate	1.0 g
Distilled water	1,000.0 ml

Heat to boiling to dissolve the medium completely. The pH should be 6.9. Add

Meta-cresol sulfon phthalein (0.4 per cent alcoholic solution)	10.0 ml

[1] This work is a joint project of the Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois, and the University of Nebraska, College of Medicine, Omaha, Nebraska.

Tube in 4-ml amounts in 10 by 1.2 cm test tubes. Sterilize in the autoclave for 15 minutes at 15 pounds' pressure. Cool and store in the refrigerator.

B. The "urea" overlaying solution,

Urea	25.0 g
Sodium chloride	5.0 g
Dipotassium phosphate	1.5 g
Monopotassium phosphate	1.0 g
Distilled water	1,000.0 ml

The pH should be 6.9. Add

Meta-cresol sulfon phthalein (0.4 per cent alcoholic solution)	10.0 ml

Sterilize by Seitz (or Berkefeld) filtration. Distribute in approximately 10-ml amounts and store in appropriate sterile screw-cap containers in the refrigerator.

C. The "urea-free" overlaying solution,

Same formula as in B, except for omission of urea. The solution may be sterilized in the autoclave, since urea is not present.

II. The test procedure:

The inoculation is made from a nutrient or Kligler's iron agar slant. A large inoculum on a straight needle is stabbed into the center of each of two tubes of buffered semisolid "deep." The surface of one is covered with 0.2 ml of urea overlaying solution. The second "deep" is overlaid with 0.2 ml of urea-free solution and serves as the control.

III. Interpretation of reactions after 18 to 24 hours' incubation at 37 C.

A. Colorimetric reaction (yellow to purple) diffusing from the surface and beyond half the length of the "deep," ++, positive.

B. Colorimetric reaction diffusing from the surface but *not* beyond half the length of the "deep," +, positive.

C. Colorimetric change at the surface of the "deep,"±, and no change in color, −, negative.

RESULTS AND DISCUSSION

Rustigian and Stuart noted pH changes as high as 9.4 for members of the genus *Proteus* cultivated in their urease test medium. The one exception was *Proteus morganii*, which attained a pH of 8.2 following 48 hours of cultivation.

The medium herein described contains the indicator *meta*-cresolsulfonphthalein, whose deepest color change occurs at pH 8.3. The buffered semisolid "deep" permits optimum growth of organisms with maximum urease production. The urea overlaying solution encourages the maximum urease activity at its optimum pH of 6.9. Positive results are usually discernible after 6 to 8 hours and are very prominent after 18 to 24 hours' incubation 37 C.

Table 1 lists 97 cultures of species of *Proteus*. All gave marked positive (++) reactions in 24 hours. Cultures of paracolon, *Shigella*, *Salmonella*, and *Eberthella typhosa* organisms all gave negative reactions. Two typical cultures of *Pseudomonas aeruginosa*, which readily produce alkalinity and actively proteolyze milk, apparently failed to attack urea. Two cultures of *Alcaligenes faecalis* and three cultures of *Escherichia coli* were urease-negative. Koser (1918) observed that *Aerobacter aerogenes* attacked uric acid as a source of nitrogen. A culture from our stock collection, typical of the species described in Bergey's manual, was examined for urease activity and found to give a definite

TABLE 1

Urease reactions of some gram-negative bacilli

CULTURE	NUMBER	NEW UREASE MEDIUM		UREA-FREE CONTROL	ANDERSON'S MODIFICATION
		6–8 hours	18–24 hours		
Shigella ambigua	2	–	–	–	–
Salmonella types*	19	–	–	–	–(10) doubtful(9)
E. typhosa	3	–	–	–	–
Paracolon species	37	–	–(36) ±(1)	–	–(36) doubtful(1)
Proteus species	74	+	++	–	+
Proteus morganii†	16	+	++	–	+
Proteus morganii (stock collection)	3	+	++	–	+
Proteus vulgaris (stock collection)	4	+	++	–	+
Alcaligenes faecalis	2	–	–	–	–
Pseudomonas aeruginosa	2	–	–	–	–
E. coli v. *communior*	2	–	–	–	–
E. coli v. *communis*	1	–	–	–	–
Aerobacter aerogene	1	–	+	–	+
Brucella abortus	1	–	+	–	+

* *S. pullorum*, 6; *S. anatum*, 2; *S. paratyphi* B, 5; *S. enteritidis*, 4; *S.* species (unidentified), 2.

† Atypical strains of *P. morganii* from an investigation in progress.

positive (+) reaction. This culture was also positive in Anderson's modified urea medium. Another interesting observation was made on a culture of *Brucella abortus*.[2] Bergey's manual states that "ammonia is produced from urea" by *Brucella melitensis* and that "the cultural characters [of *Brucella abortus*] are similar to those of *Brucella melitensis*." We noted that *Brucella abortus* attacked urea, giving a definite positive (+) reaction, whereas the control remained negative. This too was confirmed in Anderson's modified urea medium.

In general, correlation of results with Anderson's modified urea medium was particularly good for members of the genus *Proteus*. However, in our hands, a

[2] Culture obtained through the courtesy of the Seventh Service Command Medical Laboratory, Fort Omaha, Nebraska.

few cultures of *Salmonella* types were difficult to interpret at the end of the half-hour incubation interval recommended by Anderson.

The use of a nitrogenous substance, such as bacto tryptose, in the urea medium may be theoretically criticized on the basis of possible ammonia production. However, actively proteolytic and alkaline-producing organisms, such as *Pseudomonas aeruginosa* and *Alcaligenes faecalis*, that have been studied have failed to show any colorimetric changes in the test medium at the end of the 24-hour incubation period. Furthermore, of 167 cultures studied (table 1), a definite positive colorimetric change has not once been noted in the urea-free controls during the specified time interval.

SUMMARY

A new medium for the detection of urea-splitting organisms is described.

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